JOURNAL OF MEDICINAL FOOD
J Med Food 17 (10) 2014, 1064–1069

DOI: 10.1089/jmf.2013.0177

Retention of Polyphenolic Species in Spray-Dried Blackberry Extract Using Mannitol as a Thermoprotectant

Joshua A. Eldridge,[1] Debra Repko,[2] and Russell J. Mumper[1,3]

[1]*Four Tigers LLC, Lexington, Kentucky.*
[2]*Büchi Corporation, New Castle, Delaware.*
[3]*Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina.*

ABSTRACT The purpose of these studies was to determine if a Büchi Mini Spray Dryer B-290 (Büchi Corporation, New Castle, DE, USA) could be used to prepare blackberry extract powders containing mannitol as a thermoprotectant without extensively degrading anthocyanins and polyphenols in the resulting powders. Three blackberry puree extract samples were each prepared by sonication of puree in 30/70% ethanol/water containing 0.003% HCl. Blackberry puree extract sample 1 (S1) contained no mannitol, while blackberry puree extract sample 2 (S2) contained 3.0:1 (w/w) mannitol:berry extract, and blackberry puree extract sample 3 (S3) contained 6.3:1 (w/w) mannitol:berry extract. The levels of anthocyanins and polyphenols in reconstituted spray-dried powders produced from S1–S3 were compared to solutions of S1–S3 that were held at 4°C as controls. All extract samples could be spray-dried using the Büchi Mini Spray Dryer B-290. S1, with no mannitol, showed a 30.8% decrease in anthocyanins and a 24.1% decrease in polyphenols following spray-drying. However, S2 had a reduction in anthocyanins of only 13.8%, while polyphenols were reduced by only 6.1%. S3, with a ratio of mannitol to berry extract of 6.3:1, exhibited a 12.5% decrease in anthocyanins while the decrease in polyphenols after spray-drying was not statistically significant ($P = .16$). Collectively, these data indicate that a Büchi Mini Spray Dryer B-290 is a suitable platform for producing stable berry extract powders, and that mannitol is a suitable thermoprotectant that facilitates retention of thermosensitive polyphenolic species in berry extracts during spray-drying.

KEY WORDS: • ***anthocyanins*** • ***carriers*** • ***phenolics*** • ***powder*** • ***stability*** • ***thermal protectant***

INTRODUCTION

VEGETABLE AND FRUIT EXTRACTS have long been investigated for medical and health properties. These extracts have been produced using either aqueous or solvent-based extractions, and either method has been shown to lead to an extract containing many types of phenolic phytochemicals including phenolic acids, flavonoids (anthocyanins), and non-flavonoid polyphenols.[1] Recent wide-spread interest in polyphenols has resulted from scientific studies that have demonstrated that these species exhibit pharmacological properties that are important for the maintenance of human health.[2–6] Anthocyanins in particular have been shown to have many advantageous biological properties. For instance, studies in our lab have demonstrated that anthocyanin-rich blackberry extracts exhibit antioxidant, antibacterial, antiviral, anti-inflammatory, and anticancer properties.[7–12] Unfortunately, polyphenols, and especially anthocyanins, are very unstable and sensitive to pH, temperature, and other environmental conditions. The purpose of this study was to investigate a scalable process to produce anthocyanin and polyphenol-rich extracts of blackberries and to produce a stable extract-powder via spray-drying.

Although spray-drying has been used to produce various plant extract powders as functional foods, and health and medical products,[13–17] few studies have demonstrated the feasibility of retaining the physical/chemical properties of polyphenols in a spray-dried extract formulation.[18–25] The spray-drying process exposes the polyphenolic species to short durations of high temperature which can significantly reduce the levels of these important natural products.[26,27] Therefore, there remains a significant unmet need to produce stabilized extracts using spray-drying.

Our group has previously characterized various extracts from blackberries[7,12] demonstrating that ethanol extracts contain five types of anthocyanins with the primary species being cyanidin-3-glucoside (71% of total).[12] In addition, the extracts were found to exhibit temperature-dependent stability. Extracts stored at −80°C were stable for at least 90 days, but extracts stored at 25°C rapidly lost anthocyanin stability. The lack of room-temperature stability raises

Manuscript received 5 December 2013. Revision accepted 22 March 2014.

Address correspondence to: Russell J. Mumper, PhD, Chief Scientific Officer, Four Tigers LLC, 1501 Bull Lea Road, Suite 101, Lexington, KY 40511, E-mail: mumper@email.unc.edu

significant concerns about the ability to develop various health and medical products containing the extracts for storage at room temperature. As a consequence, our group investigated the ability to create room-temperature stable powders by incorporating various cryoprotectants to stabilize anthocyanins and other polyphenols during lyophilization. In fact, our studies demonstrated that freeze-dried blackberry extract containing 10.46:1 w/w mannitol:berry extract exhibited no change in the level of cyanidin-3-glucoside for at least 8 weeks of storage at 25°C, and we have observed similar thermal protection with only two parts mannitol as well.[28,29] The presence of mannitol also served to eliminate the hygroscopic properties of neat freeze-dried blackberry extract.

The exact mechanism by which mannitol stabilizes anthocyanins and other polyphenols in a dry powder matrix has not been specifically investigated at this time; however, it was hypothesized that several properties of mannitol could contribute to the stabilization. Specifically, mannitol is a simple straight-chain sugar containing six hydroxyl groups per molecule, and these hydroxyl groups exhibit more degrees of freedom than the hydroxyl groups present in a cyclic sugar structure (*i.e.* maltodextrins). Accordingly, mannitol may efficiently create hydrogen bonds with the phenol groups of polyphenolic species that would, in turn, lessen the likelihood of hydrogen abstraction from phenol functional groups by oxygen (oxidation). Additionally, it was observed that mannitol-containing berry extract formulations are far less hygroscopic than neat berry extracts. This physiochemical property may assist in stabilization by lowering water activity and reducing the molecular mobility of polyphenolic species. Water activity is known to effect the rate of degradation of polyphenols.[20,23] Mannitol has also been identified as a scavenger of reactive oxygen species, including hydroxyl radicals.[30] Therefore, it was postulated that mannitol had a stabilizing effect by reducing the oxidation and molecular mobility of polyphenols; both of which are rate-dependent on temperature.

In recognizing that lyophilization was not likely to be an economical or scalable process to create room temperature-stabilized powders, we turned to spray-drying as an alternative. The current studies investigated spray-drying as a more economical and scalable manufacturing process to prepare blackberry extract powders for commercial products and also investigated whether mannitol could serve equally well as a thermoprotectant during the spray-drying process. These studies demonstrate that mannitol may serve as a suitable thermoprotectant during the spray-drying process, because it stabilizes both anthocyanins and polyphenols in a concentration-dependent manner in the resulting spray-dried powders. To our knowledge, the use of mannitol as a thermoprotectant/carrier of spray-dried fruit extract formulations has not previously been demonstrated. These findings open the possibility to produce commercial quantities of a stable fruit-extract that may be subsequently incorporated into various room-temperature stable dosage forms.

MATERIALS AND METHODS

Blackberry Puree was obtained from Chester blackberries grown at Windstone Farms (Paris, KY, USA). Folin-Ciocalteau phenol reagent, gallic acid (98%), and 1.25 M HCl in ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). USP grade ethanol (200 proof) was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY, USA). USP-grade mannitol was obtained from Fisher Scientific (Waltham, MA, USA).

Preparation of Chester blackberry puree extracts

During the Kentucky summer harvest season, Chester blackberries from Windstone Farms were picked from 10:00 AM to 2:30 PM, August 21, 2013, and washed with 0.1 cup bleach/150 gallons tap water[31] from Bourbon County, Kentucky. The adult Chester blackberry plants were 7 years old at the time of the harvest. Blackberries were immediately frozen after washing and stored frozen until puree processing. All berries were thawed overnight at 4°C and hand pureed the next day with a food mill and a #14 mesh flour sifter into a clean five gallon bucket. The latter process removed skins and seeds. Puree was not dried before further use. For each sample (S1–S3), 200 g of aqueous puree was extracted with 820 mL of 30/70% ethanol/water (0.003% HCl) in a 1 L Erlenmeyer flask. The solvent system consisted of 250 mL ethanol (0.01% HCl) mixed with 570 mL distilled water. Since the puree matrix in these extractions is known to be 90% water (180 mL water), the final composition of extraction mixtures in S1–S3 was 20 g blackberry material plus 1 L of 25/75% ethanol/water (0.0025% HCl). These extraction mixtures were extracted and filtered to yield identical extracts before mannitol was added to S2 and S3 in the final formulation step. Extractions were carried out for 30 minutes each in a chilled water bath using ultrasonic cavitation. Afterward, the pulp and liquid extract from each sample were divided into individual 50 mL centrifuge tubes to spin down the pulp mass at 3000 RPM for 15 minutes. Approximately 800 mL extracts were recovered by vacuum filtration over Whatman Grade 4 qualitative filter papers (Sigma-Aldrich, St. Louis, MO, USA); however, there was some variation in the amount of filtrate (extract) recovered between S1–S3. Finally, S1 was not spiked with mannitol after filtration while S2 and S3 were spiked with 20.002 and 40.003 grams of mannitol, respectively. Actual solute concentrations in the final solutions (S1–S3) were determined by gravimetric analysis after carefully evaporating the solvent to leave a fixed mass of solute. S2 and S3 were assayed for their solute ratios (mannitol:blackberry extract) by using a comparison of the levels of total phenols in S1 to those in S2 and S3.

For spray-drying, 200 mL aliquots were removed from S1–S3, and all samples were stored at 4°C overnight and then shipped by overnight delivery to the Büchi Corporation on ice packs in a foam cooler. Upon arrival at Büchi, the samples were placed in a refrigerator at 4°C until spray-dried the next day. Samples were spray-dried and then sent back to Lexington, KY, for determination of monomeric

anthocyanins and total phenols as described below. In all, there were a total of nine days from original sample extraction to anthocyanin and phenol assays. Except during spray-drying time, all extract samples and controls remained at 4°C.

Spray-drying set-up

The spray-dryer equipment consisted of a Büchi Mini Spray Dryer B-290 Advanced 230 V/50–60 Hz unit coupled to a Büchi Dehumidifier B-296, 230 V/50–60 Hz. A polyester membrane filter was installed in the system to filter the outlet gas. The standard glassware, which came with the instrument and consists of a cylinder with separation flask, a standard cyclone separator, and a collection flask, proved to work most effectively. The instrument was set up on a stainless-steel trolley for ease of moving when necessary. Compressed nitrogen was used as an atomizing gas while conditioned room air served as the inlet drying gas. Solutions (200 mL) of S1–S3 were fed to the B-290 at a rate of 7–9 mL/min with an average processing time of 30 minutes each.

Determination of solute concentration in S1–S3 by gravimetric analysis

From each extract sample S1–S3, three separate aliquots of 1 mL were removed by pipet and transferred to preweighed vials. The samples were stripped of solvent to a constant weight, and the average final weights of S1–S3 were used to determine their solute concentrations (mg/mL).

Determination of monomeric anthocyanins levels

Total anthocyanins were determined using the pH-differential method of Giusti and Wrolstad.[32] From each extract sample held at 4°C (S1–S3), there were removed six 100 μL aliquots into separate cuvettes of approximately 2.5 mL capacity. The solutions in three cuvettes of each sample (S1–S3) were diluted with 900 μL of pH 1.0 buffer (0.025 M KCl). The solutions in the other three cuvettes from each sample were diluted with 900 μL of pH 4.5 buffer (0.4 M sodium acetate). For spray-dried samples of S1–S3, three separate samples per group were weighed and reconstituted in exactly 1 mL of 25/75 ethanol/water (.0025% HCl) in amounts that would ensure the same concentrations as S1–S3 extracts (10 mg, 36 mg, and 63 mg, respectively). Each reconstituted sample was assayed as described above ($n = 9$). A Beckman Coulter DU® 720 General Purpose UV/Vis Spectrophotometer (Brea, CA, USA) was used to measure the absorbance values at pH 1.0 and pH 4.5 compared to a distilled water blank using wavelengths of 510 nm and 700 nm. Total anthocyanin content was calculated using an extinction coefficient of 26,900 L cm^{-1} mol^{-1} and a molecular weight of 449.2 g mol^{-1} of cyanidin-3-glucoside.

Determination of total phenols

Total phenols were determined using the Folin-Ciocalteau method.[33] Standards of 0, 200, 400, 500, 800, and 1000 mg/L gallic acid were prepared from a stock solution (5 g/L). Aliquots of 20 μL were removed from the standards and S1–S3 extracts. The aliquots were then diluted with 1580 μL distilled water. Folin-Ciocalteau reagent (100 μL) was added to each cuvette and mixed well. Within 30 seconds to 8 minutes, each sample was treated with 300 μL of saturated sodium carbonate solution. The samples were left to stand for 2 hours before analysis ($n = 3$). Spray-dried samples were reconstituted in the original extract solvent system as described under the section titled ''Determination of monomeric athocyanins levels'' and assayed as $n = 9$ samples. A Beckman Coulter DU 720 General Purpose UV/Vis Spectrophotometer was used to measure the absorbance values compared to a blank analytical standard ($\lambda = 765$ nm). Sample concentrations of phenols were calculated from the least-squares regression line of the analytical standards.

Determination of percent yield of spray-dried powder

Solute concentrations determined from gravimetric analysis were used to calculate percent yield numbers based on spray-dried volumes and theoretical yields.

Statistical analysis

All values of each assay were based on independent triplicate samples of S1–S3 extracts and $n = 9$ samples for spray-dried powders. Data are reported as the mean ± SD. Statistical analysis was performed using Student's paired *t*-tests. A *P*-value of $P \leq .05$ was considered significant.

RESULTS

The solute concentrations of S1–S3 were found to be 10, 36, and 63 mg/mL, respectively. Spray-dryer parameters that were used in the study are reported in Table 1. One hundred and fifty mg of product was obtained from 200 mL of S1 extract ($\sim$7.5% yield), and the recovered material was very hygroscopic and clumpy. Anthocyanins levels were reduced by 30.8% and phenols levels were lowered by 24.1% in spray-dried S1 compared to the original extract (Table 2). From approximately 150 mL of the S2 extract formulation, 0.59 grams of product (10.9%) was obtained as a pink solid with fairly large particle sizes. Mannitol exhibited a thermoprotective effect during spray-drying as the anthocyanins levels were only lowered by 13.8% in S2 (Table 2). Polyphenols were more resilient to the spray-drying process with only 6.1% decrease compared to the original S2 extract formulation (Table 2). The total phenols assay was used to determine the percent decrease of phenols in the S2 extract formulation (before spray-drying) due to mannitol dilution as compared to the level of phenols in the S1 control extract. It was found that the weight ratio of mannitol:berry extract in S2 was 3.0:1. Using the same methodology for S3, a 6.3:1 mannitol:berry extract ratio was established. S3 showed the best spray-drying characteristics with a 61.3% (7.72 g) yield (Table 1) of pink free-flowing powder obtained from approximately 200 mL of solution and a percent decrease of anthocyanins of 12.5% (Table 2).

Table 1. Büchi Mini Spray Dryer B-290 Parameters and Recovery of Extract

Sample ID	*Inlet T (°C)*	*Outlet T (°C)*	*Aspirator %*	*Sample pump %*	*Air flow rate (mm)*	*% Yield*
S1	150	72	100	25	40	7.5
S2	160	56	89	30	40	10.9
S3	150	75	100	30	40	61.3

Individual parameters of spray-dried powders of three blackberry puree extract samples (S1–S3) prepared by a Büchi Mini Spray Dryer B-290 with standard glassware coupled to a B-296 Dehumidifier apparatus. Sample runs were approximately 200 mL per sample with an average processing time of 30 minutes per sample.

Furthermore, there was no significant decrease of phenols measured in spray-dried samples of S3 compared to the S3 extract formulation. These data suggest that mannitol serves as a suitable thermoprotectant for blackberry extract samples, and that the levels of anthocyanins and other polyphenols are adequately preserved by the presence of mannitol in the spray-drying formulation.

DISCUSSION

In these studies, spray-dried powders of blackberry extracts showed good retention of polyphenolic species when mannitol was utilized as a thermoprotectant. In particular, S3 demonstrated a recovery of 61.3% material that is within the expected range of the spray-drying process using the B-290 and relatively small volumes of spray-drying liquids. In comparison to larger scale attempts by other groups, similar yields (67%) to S3 were previously reported by Yatsu *et al.* at semi-industrial scale using a silicon dioxide carrier and a Niro Production Minor™ Spray Dryer (GEA Niro, Søborg, Denmark) to prepare powders of *Ilex paraguariensis.* However, stability of polyphenols in the powders at 40°C and 75% relative humidity was relatively poor over a 4-month time course.[34] In the current study, the lower mannitol concentration in the S2 spray-drying formulation was also effective at reducing the loss of polyphenolic species due to thermal processing procedures, but the yield of S2 suggested the need for further formulation and instrument optimization work.

Surprisingly, the control extract (S1) exhibited a loss of only 30.8% anthocyanins with a 24.1% decrease of total phenols. These findings indicate that more aqueous-based extraction solvent systems, such as 25/75% ethanol/water (0.0025% HCl), may extract thermoprotective sugars from the blackberry puree. Testing this hypothesis continues in on-going studies.

Fernández-López *et al*. recently reported temperature-dependent rates of color degradation in anthocyanin-rich extracts isolated from elderberry, red cabbage, hibiscus, and cochineal.[35] Elderberry exhibited the best thermal stability among the anthocyanin-containing botanical extracts while cochineal extract was the most heat stable of the samples analyzed. Ferrari *et al.* demonstrated that blackberry juice formulations containing maltodextrin and gum arabic carriers could be spray-dried and stored with a half-life for total anthocyanins of about 9.9–12.5 months at 25°C depending on the carrier.[21,22] Similarly, spray-drying with maltodextrin 10DE carrier and subsequent cold storage was found by Fang *et al.* to be an appropriate combination for retention of polyphenolic species in bayberry juice[20] while Tonon and others also found maltodextrin 10DE to be the best of four different carriers utilized for spray-dried acai juice powder under various conditions of temperature and water activity.[23] In another study by Obón *et al*., glucose syrup (Glucidex® Dehydrated Glucose Syrup 29, Roquette America, Geneva, IL, USA) in combination with *Opuntia stricta* fruit juice preserved betacyanin pigments in spray-dried powders with colorimetric stability for one month at 4°C.[36] Betacyanins are indole conjugates that are dissimilar to anthocyanins and polyphenols in structure, yet they contain a phenol moiety and possess thermal sensitivity. Puree and ethanolic extracts of prickly pear cactus were spray-dried by Saénz *et al*. using maltodextrin and inulin as carriers.[24] In the latter study, stable betacyanins were 100% recovered from puree with both carriers while variable results were observed when

Table 2. Levels of Anthocyanins and Polyphenols in Solute Before and After Spray-Drying

Sample ID (M:BE)[a]	*Anthos*[b] *(mg/g) before SD*	*Anthos*[b] *(mg/g) after SD*	*% Decrease anthos*	*Phenols*[c] *(mg/g) before SD*	*Phenols*[c] *(mg/g) after SD*	*% Decrease phenols*
S1 (*0:1*)	11.7 ± 0.1	8.1 ± 0.1*	30.8	39.8 ± 0.3	30.2 ± 2.5*	24.1
S2 (*3.0:1*)	2.9 ± 0.0	2.5 ± 0.0*	13.8	9.9 ± 0.1	9.3 ± 0.2*	6.1
S3 (*6.3:1*)	1.6 ± 0.0	1.4 ± 0.0*	12.5	5.4 ± 0.0	5.3 ± 0.1	1.9

[a]M:BE is the weight ratio of mannitol (M) to berry extract (BE) composition in solute.

[b]Total anthocyanins were expressed as cyanidin-3-glucoside equivalent.

[c]Total phenols were expressed as gallic acid equivalent.

*Signifies that there is statistical significance ($P < .05$) between the sample obtained after spray-drying (''after SD'') compared to the sample before spray-drying (''before SD'').

Data reported as mean ± standard deviation, $n = 3$ in extracts before spray-drying, $n = 9$ in spray-dried powders.

maltodextrin was used as the carrier to spray-dry ethanolic extracts. Ersus *et al*. showed that ethanolic extracts of black carrot exhibited approximately 59–77% retention of anthocyanins (accounting for carrier dilution) after spray-drying using three different maltodextrins.[25] Therefore, there is prior evidence for the use of maltodextrin carriers for thermal protection during the spray-drying of formulations containing thermosensitive chemical species such as anthocyanins. However, as illustrated above, maltodextrins have shown variable results in protecting polyphenolic species from thermal degradation. To our knowledge, few other possible thermoprotectant carriers have been investigated for spray-drying extracts.

In conclusion, we have demonstrated that mannitol can serve as a thermoprotectant in spray-dryer processing procedures that are intended to preserve polyphenolic plant phytochemicals. Powders produced in this way hold promise for the development of functional foods and medical products. The current literature indicates that maltodextrins and a few other carrier agents have been utilized in studies of this type. However, mannitol appears to have been overlooked as a thermoprotectant of plant-derived powders that contain easily oxidized and thermally unstable chemical species. Our results indicate that mannitol provides thermal protection to delicate polyphenols in a concentration-dependent manner, and that the protection is comparable or superior to many of the results observed with other spray-drying carrier agents, including maltodextrins. The findings of these studies warrant further investigation of the use of mannitol as a thermoprotectant in other polyphenol-containing fruit or vegetable extracts that may benefit from or require spray-drying to manufacture powders for incorporation into various health or medical products.

ACKNOWLEDGMENTS

Funding for the study was provided by Kentucky Science and Technology Corporation Award #KSTC-17-OCIS-183 through Kentucky's SBIR-STTR Matching Funds Program. Research was also partially supported by the National Institute of Dental & Craniofacial Research of the National Institutes of Health (NIH) via R42DE018839. The content is solely the responsibility of the authors and does not represent the official views of the NIH.

AUTHOR DISCLOSURE STATEMENT

Joshua A. Eldridge is an employee of Four Tigers LLC. Russell J. Mumper is founder and Chief Scientific Officer of Four Tigers LLC, and has an equity interest in the company. Debra Repko is an employee of the Büchi Corporation and has no equity interest in Four Tigers LLC.

REFERENCES

1. Dai J, Mumper RJ: Plant phenolics: extraction analysis and their antioxidant and anticancer properties. *Molecules* 2010;15:7313–7352.
2. Beattie J, Crozier A, Duthie GG: Potential health benefits of berries. *Curr Nutr Food Sci* 2005;1:71–86.
3. Paredes-López O, Cervantes-Ceja ML, Vigna-Pérez M, Hernández-Pérez T: Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life–A Review. *Plant Foods Hum Nutr* 2010;65:299–308.
4. Lila MA: Anthocyanins and human health: An *in vitro* investigative approach. *J Biomed Biotechnol* 2004;5:306–313.
5. Andújar I, Recio MC, Giner RM, Ríos JL: Cocoa polyphenols and their potential benefits for human health. *Oxid Med Cell Longev* (Online) published May 2012, 10.1155/2012/906252.
6. Vauzour D, Rodriguez-Mateos A, Corona G, Oruna-Concha MJ, Spencer JP: Polyphenols and human health: prevention of disease and mechanisms of action. *Nutrients* 2010;2:1106–1131.
7. Dai J, Patel JD, Mumper RJ: Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *J Med Food* 2007;10:258–265.
8. Danaher RJ, Wang C, Dai J, Mumper RJ, Miller CS: Antiviral effects of blackberry extract against herpes simplex virus type 1. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011;112:e31–e35.
9. Mallery SR, Stoner GD, Larsen PE, Fields HW, Rodrigo KA, Schwartz SJ, *et al.*: Formulation and *in-vitro* and *in-vivo* evaluation of a mucoadhesive gel containing freeze dried black raspberries: implications for oral cancer chemoprevention. *Pharm Res* 2007;24:728–737.
10. González OA, Escamilla C, Danaher RJ, Dai J, Ebersole JL, Mumper RJ, *et al.*: Antibacterial effects of blackberry extract target periodontopathogens. *J Periodontal Res* 2013;48:80–86.
11. Murapa P, Dai J, Chung M, Mumper RJ, D'Orazio J: Anthocyanin-rich fractions of blackberry extracts reduce UV-induced free radicals and oxidative damage in keratinocytes. *Phytother Res* 2012;26:106–112.
12. Dai J, Gupte A, Gates L, Mumper RJ: A comprehensive study of anthocyanin-containing extracts from selected blackberry cultivars: Extraction methods, stability, anticancer properties, and mechanisms. *Food Chem Toxicol* 2009;47:837–847.
13. Tissen C, Woertz K, Breitkreutz J, Kleinebudde P: Development of mini-tablets with 1 mm and 2 mm diameter. *Int J Pharm* 2011;416:164–170.
14. Markvicheva EA, Antonov EN, Popova AV, Bogorodskii, Likhareva VV, Fel'dman BM, *et al.*: Biodegradable polymer microparticles with entraped herbal extracts: preparation with supercritical carbon dioxide and use for tissue repair. *Biomed Khim* 2009;55:479–488.
15. Spaniol B, Bica VC, Ruppenthal LS, Volpato MR, Petrovick PR: Compressional behavior of a mixture of granules containing high load of Phyllanthus niruri spray-dried extract and granules of adjuvants: comparison between eccentric and rotary tablet machines. *AAPS PharmSciTech* 2009;10:1013–1023.
16. Soares LAL, Gonzalez OG, Petrovick PR, Schmidt PC: Dry granulation and compression of spray-dried plant extracts. *AAPS PharmSciTech* 2005;6:E359–E366.
17. Velziene S, Kalveniene Z, Aleknaviciene B, Vaiciuleniene J, Savickas A, Masteikova R: Technology for tablets with herbal extracts and evaluation of its quality. *Medicina (Kaunas)* 2003;39 Suppl 2:122–126.
18. Marquele FD, Stracieri KM, Fonseca MJV, Freitas LAP: Spray-dried propolis extract. I: physicochemical and antioxidant properties. *Pharmazie* 2006;61:325–330.

19. Berté KAS, Beux MR, Spada P, Salvador M, Hoffman-Ribani R: Chemical composition and antioxidant activity of yerba-mate (*Ilex paraguariensis* A.St.-Hil., Aquifoliaceae) extract as obtained by spray drying. *J Agric Food Chem* 2011;59:5523–5527.
20. Fang Z, Bhandari B: Effect of spray drying and storage on the stability of bayberry polyphenols. *Food Chem* 2011;129:1139–1147.
21. Ferrari CC, Germer SPM, Alvim ID, Vissotto FZ, Mauricio J: Influence of carrier agents on the physicochemical properties of blackberry powder produced by spray drying. *Int J Food Sci Technol* 2012;47:1237–1245.
22. Ferrari CC, Marconi GSP, Alvim ID, Mauricio J: Storage stability of spray-dried blackberry powder produced with maltodextrin or gum arabic. *Drying Technol* 2013;31:470–478.
23. Tonon RV, Brabet C, Hubinger MD: Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpe oleracea Mart.*) juice produced with different carrier agents. *Food Res Int* 2010; 43:907–914.
24. Saénz C, Tapia S, Chávez J, Robert P: Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus-indica*). *Food Chem* 2009;114:616–622.
25. Ersus S, Yurdagel U: Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota L.*) by spray drier. *J Food Eng* 2007;80:805–812.
26. Gancel AL, Feneuil A, Acosta O, Peréz AM, Vaillant F: Impact of industrial processing and storage on major polyphenols and the antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus*). *Food Res Int* 2011;44:2243–2251.
27. Wu R, Frei B, Kennedy JA, Zhao Y: Effects of refrigerated storage and processing technologies on the bioactive compounds and antioxidant capacities of 'Marion' and 'Evergreen' blackberries. *LWT - Food Sci Technol* 2010;43:1253–1264.
28. Mumper RJ, Dai J, Gallicchio VS: Berry preparations and extracts. U.S. Patent 7,964,223. June 21, 2011.
29. Mumper RJ, Dai J, Gallicchio VS: Berry preparations and extracts. U.S. Patent 8,367,126. February 5, 2013.
30. Shen B, Jensen RG, Bohnert HJ: Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol* 1997;115:527–532.
31. The City of Paris ''Thoroughbred Capital of the World.'' www.paris.ky.gov (accessed November 2013).
32. Giusti MM, Wrolstad RE: Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: *Current Protocols in Food Analytical Chemistry* (Wrolstad RE, Acree TE, An H, *et al.*, eds.), John Wiley & Sons, New York, 2001.
33. Singleton VL, Rossi JA: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965;16:144–158.
34. Yatsu FKJ, Borghetti GS, Basani VL: Technological characterization and stability of *Ilex paraguariensis* St. Hil. Aquifoliaceae (Maté) spray-dried powder. *J Med Food* 2011;14:413–419.
35. Fernández-López JA, Angosto JM, Giménez PJ, León G: Thermal stability of selected natural red extracts used as food colorants. *Plant Foods Hum Nutr* 2013;68:11–17.
36. Obón JM, Castellar MR, Alacid M, Fernández-López JA: Production of a red–purple food colorant from Opuntia stricta fruits by spray drying and its application in food model systems. *J Food Eng* 2009;90:471–479.